PNEUMONIC PLAGUE

KARL F. MEYER

George William Hooper Foundation, University of California Medical Center, San Francisco, California

The primary pneumonic plague outbreaks in Oakland in 1919 and Los Angeles in 1924 constitute good points of reference for epidemiological observations and experimental studies on this form of plague. Retrospective analysis of them reveals some still unanswered questions.

In the Oakland outbreak (24), a man went hunting in the hills of Contra Costa County in April 1919 and perhaps took home a squirrel for food. He fell ill about 4 days later, having an axillary bubo and a secondary pneumonia. Another man living in the same house contracted plague and in turn passed it directly or indirectly to 12 others. All of the patients died, plus two physicians and two nurses. Confusing clinical and bacteriological observations raise some doubt that plague was the cause of death of one of the nurses. The disease was first taken to be influenza, but the diagnosis was changed after bipolar bacilli were seen in smears from the lung and after animal inoculation tests in one case. Plague was not found among 6,000 rats trapped soon afterward, but infected squirrels were discovered in the area where the man had hunted. The weather was warm and dry.

As reported on the Los Angeles outbreak (9), in October 1924 a Mexican woman died after an illness of 4 days. Her husband and nurse became ill and both died. The cause of death of the husband was given as "double lobar pneumonia." Within 2 days, 32 cases of pneumonic plague, all fatal, were discovered in persons living in the house and in friends and relatives who had visited the home of the first patient, who probably had had a secondary plague pneumonia. Among them were four children 10 years old or less, one woman of 50, a priest, a nurse, and an ambulance driver who helped transport the patients. Bubonic plague had been diagnosed in other patients in the area, and plague bacilli were found in commensal rats that had in all probability contracted it from squirrel fleas. The climate was not unusual.

The clinical histories, autopsy reports, and cultures of the plague strains isolated from ten of the victims did not indicate where the first patient contracted the infection nor precisely how it spread.

Similar epidemics had been observed in India (50) and in Manchuria in 1910-1911 and 1920-1921. According to the classical account of Sticker (56, 57), pneumonic plague was first seen to be associated with bubonic plague in the 14th century. In few accounts is it clear whether the pneumonia was primary or secondary and to what extent it was contagious. By the middle of the 16th century it was noticed that pneumonic outbreaks were preceded by bubo pest.

From the earliest time the question of how plague spreads has been the foremost one. At first this was a very simple question, and for lack of specific information the principle of isolation was applied if possible. So far as the pneumonic form is concerned, the question remains largely unanswered even though many empty spaces in general knowledge of plague and its spread, especially the bubonic form, have been filled.

In a valuable collection of letters at the University in Basel, Switzerland, between the humanists and Paracelsists dealing with plague in 1563-1564, a significant, simple, still tenable concept of spread is spelled out (23). J. Crato, in a letter dated 1565 and addressed to Theodor Zwinger, wrote that transmission of pneumonic plague is due to inhalation of plague seminaria in the expired air of plague patients who exhale the plague contagium and disperse it in the air. The significance of the concept, that pneumonic plague is airborne, was not realized because in the depopulating epidemics that gave it the name of Black Death, pneumonia was subordinate in the spread of plague in Europe. Procedures to test this concept became available only during the most recent pandemic and the great Manchurian pneumonic plague epidemics of 1910-1911 with 60,000 deaths. Their extreme infectivity under climatic and living conditions favorable to spread are well documented (68). The pneumonic form was first bacteriologically proved by Childe (6) in cases in Bombay in 1896.

Strong and Teague (58) and Strong, Crowell,

and Teague (59) furnished the first experimental evidence to support Crato's theory of spread. After exposing plates of agar through the wards in the neighborhood of patients and before patients during and between coughing spells, they saw that more plague bacilli were disseminated into the air during coughing than during talking or breathing. They did not measure the maximal distance but estimated that droplets might be propelled several yards. Little was known 50 years ago about the behavior of airborne droplets or the existence of droplet nuclei. Variation in the humidity of the air was recognized as a factor, and the effects of atmospheric dryness and cold were seen in experiments. Cold, high relative humidity, and the presence of mucus in sputum were believed to prevent droplets from drying or settling quickly. At the International Plague Conference (21) in Mukden in 1911, where these findings were reported, the conclusion was reached that since in many cases transmission has followed close approach to or actual contact with a patient and since the wearing of an efficient antimicrobial mask protects, pneumonic plague is an aerial infection.

Some disagreed, believing that plague bacilli are introduced orally, through kissing or with fingers soiled with feces of plague-infected fleas or during handling of plague-infected wild rodents. Laboratory infections, for example, in the Allgemeine Krankenhaus in Vienna, supported this idea. An animal caretaker handling guinea pigs with cutaneous lesions contracted pneumonic plague, which rapidly spread to his physician and then the physician's nurse (36), as did the Los Angeles outbreak. It was thought to have been the funeral of the first victim that thoroughly saturated these demonstrative people with *Pasteurella pestis*. Dust contaminated with the feces of plague-infected rodents or bedding soiled with the sputum of pneumonic plague patients was believed to produce primary lung infection. True, direct contact may spread the infection, but the air seems to be the usual conveyor of pneumonic plague among human beings.

Although this infection has an unequaled reputation for contagion, there have been circumstances in which patients with secondary pneumonia or with pneumonic plague neither initiated nor continued epidemics. Why an occasional patient with a terminal pneumonia suddenly begins to infect contacts approaching him or breathing the same air also remains a puzzle; perhaps exhalation of the organisms is not constant. Furthermore, the early studies on plague by the Indian Plague Commission (20) were explicit in indicating that the relationship between inhalation of plague bacilli into the respiratory tract and the supervention of primary plague pneumonia is not necessarily direct.

Some unusual character of the infecting organism was suspected to be responsible for the pneumonic character; during this immediate postmicroscopic period the organism was the major preoccupation. Neither serological nor cultural differences that would enable prediction of clinical effects have been detected among plague strains. Enhanced specific tropism for lung tissue cannot be revealed by any known technique. Secondary lung manifestations are by no means usual in animals inoculated percutaneously or subcutaneously with strains freshly isolated from the sputum or lung tissue from pneumonic plague patients; guinea pigs have succumbed quickly, showing septicemia and moderate adenopathy within 3 to 4 days. Freshly isolated strains from bubonic plague patients have had the same effects. On the other hand, almost all rodents infected cutaneously with plague bacilli, whether from pneumonic or bubonic plague, show disseminated foci of bronchopneumonia when they survive longer than 6 days. They show tendencies to pulmonary localization even if the skin is merely denuded and not scarified; this is true particularly when the issue of the fatal infection is delayed (8). Neither exalted virulence nor pneumotropism accounted for the characteristics of the Oakland and Los Angeles episodes.

Another explanation was that the strains from wild rodents were particularly likely to cause secondary lung involvement. The epidemiological evidence, however, is not convincing. In the United States, except for the two mentioned outbreaks, pneumonic plague has not been observed. Most cases of wild rodent origin have occurred singly, and secondary pulmonary complications have been inconspicuous. Furthermore, pneumonic plague is the usual form in Madagascar and Indonesia, where ordinary domestic rats form the reservoir.

With the introduction of the biotest, the use of flea inoculations, to locate wild rodent plague foci

in California, it was noted that macroscopic lung lesions were not uncommon in test animals. At first this observation was considered significant, but it was soon observed that plague bacilli which resided in domestic fleas produced lung lesions as readily as those in wild rodent fleas. This was particularly noticeable in guinea pigs which died after the sixth day. On artificial cultivation, this apparent pneumotropism was lost except when the dose was reduced and the disease in the guinea pigs was prolonged.

Investigators of the Oakland epidemic first thought it to be a highly virulent form of influenza. This recalled the idea of certain Indian plague workers, the German Plague Commission (14) in 1898 and 1907, and Calmette and Salimbeni (5) that some visible or ultramicroscopic virus might be associated with *P. pestis* and that this mixed infection might account for the pneumonic type.

The physician who had seen the patient in Oakland who was the crux of the outbreak had made the diagnosis "influenza." There were still residuals of pandemic influenza in the early months of 1919; but since it had disappeared by the time of the epidemic, the cases of fatal so-called "influenza" aroused doubts among the health officials. At that time, procedures for conclusive diagnosis of viral respiratory infections had not yet been devised. The incidence of respiratory infection in Los Angeles in October 1924 was low and not exceptional in the Mexican population in the area around the plague focus. Particular attention was paid to this since in the years just before White (63) and Nicolle and Gobert (40) had insisted on a close connection between influenza and pneumonic plague. However, extensive epidemiological observations lend little support to the idea that other pathogens encourage the development of pneumonic plague. Seasonal occurrence of influenza in Madagascar did not increase pulmonary complications in plague outbreaks. McCrumb and his colleagues (34) failed to obtain serological evidence that influenza viruses were involved in the pathogenesis of the cases of pneumonic plague they studied in Madagascar. Perhaps viruses other than influenza virus might make pneumonic plague highly infectious (54) in the rare case in which they coexist, but it would seem unwise to doubt the infectiousness of pneumonic plague alone. The house epidemics described by Tieh and others (60) illustrate this point. Of course the same factors may encourage the development and spread of respiratory infection in general.

Pneumonic epidemics, then, do not seem to be attributable to any peculiar property of the bacillus, to any particular rodent or flea species, nor to mixed infection. Their spread depends more on extrinsic factors which bring the parasite and host together than on intrinsic factors in either.

It seems more reasonable to assume their origin in a source that usually has disappeared by the time the pneumonic outbreak erupts, i.e., a patient with severe, prolonged bubonic plague who has full-blown secondary pneumonia, who coughs and expectorates copiously, and who behaves like the "cloud baby" recently described in staphylococcal infections (12). Epidemiological inquiry may be crippled by the circumstance that no stable witnesses survive to give information about this patient. Pathological investigations may be handicapped because in many areas autopsy is not permitted. The few cases in which the clinical course could have been observed took place before the development of precise methods of measurement and observation.

In the Oakland outbreak, one salient point has not been emphasized, namely, that a physician called in consultation, impressed by the painful axillary swelling, had incised and then reopened it on two occasions within 4 days. The surgical manipulations of the axillary bubo may have detached thrombi loaded with bacilli and these may have become emboli in the lung.

In the former German colony in West Africa, a patient coming down with bubonic plague traveled from infected Dar es Salaan into the interior to his home village, stopping and talking with villagers along the way. During the first 3 days he passed through four villages, through two settlements on the fourth and fifth days, and finally reached his home village, where he died. Eleven cases of primary pneumonic plague marked his trail, eight contracted on the fourth and fifth days of his journey, and three in his village (K. F. Meyer, *personal observation, unpublished*).

Investigators of the Manchurian epidemic traced outbreaks to a traveler with secondary plague pneumonia proceeding from an infected to an uninfected place. Again in 1946, the epi-

demic described by Tieh and other observers (60) in Mukden began with a visit.

Petrie and Todd (42), while studying plague in Egypt, also noticed that travelers are particularly likely to develop marked secondary lung involvement. They suggested the interesting explanation that in the dry desert a high saturation deficiency of the air causes evaporation of moisture from the pulmonary mucous membrane, disposing it to secondary pneumonia.

Just 2 years ago in Madagascar another traveling plague patient distributed the organisms which caused an epidemic (4).

Once the news of a plague epidemic circulates, the usual reaction is flight, an old problem in plague control, and the dying victims may want to return home. Some of those who flee are already infected but have no symptoms. There are undoubtedly factors in this flight effort, such as exposure, exhaustion, and change of environment, that might aggravate the illness and favor respiratory complications. There is no detailed knowledge of this patient: whether excretion of plague bacilli takes place in all cases, whether this excretion is intermittent, or of the true nature of the responsible exposure. Corresponding facts are not known of the recipient: the minimal infective dose, susceptibility, exposure, and climatic conditions which might favor respiratory infections. Social customs participate, especially those concerning reunion, impending death, death, and interment.

All the more serious outbreaks reported have originated under circumstances particularly favorable to infections through the respiratory tract. In the Manchurian epidemics, gross overcrowding in badly ventilated underground inns with tightly shut doors and windows probably charged the air with great concentrations of droplets of microbe-laden moisture ejected from the lungs during coughing. Strong and associates (58, 59) mentioned that the cloud of condensed vapors issuing from the mouth during expiration was seen to extend a distance of 30 cm. During the International Plague Conference in 1911, it was recognized that pneumonic plague is contracted indoors most commonly.

That pestilential air is a vehicle of infection was known long before microorganisms could be seen. By analogy with other respiratory infections, the inhalation airborne disease theory was adopted for pneumonic plague without consideration of the concept of infectious droplets advanced by Flügge at the beginning of this century. One group of epidemiologists and pathologists insisted that *P. pestis* is directly inhaled into the air passages. Others argued that the primary infection is in the tonsils and that the lungs are only secondarily infected by bacilli carried by the blood. The first group reasoned that severe pneumonic plague is caused by entry of the organisms through the lower parts of the respiratory tract. There is an element of truth on both sides.

Plague seems to be contracted in many cases when the infectee comes within close range of the coughing infector. Since the frequency of cough and the quantity of bacilli sprayed undoubtedly vary from case to case, and probably from time to time in the same case, depending on the nature and severity of the respiratory involvement, the infectiousness of pneumonic plague patients also probably varies greatly. Early in the infection, when the patient coughs little and plague bacilli in the expectorations are few or even absent, he is not likely to be highly dangerous.

Patients, suffering from a confusing form of septicemic or bubonic plague acquired through the respiratory tract, in which cough is an insignificant symptom, are not infectious. This was first mentioned in 1922 when Wu Lien-Teh, Chun, and Pollitzer (67), reported on 34 autopsies of victims of plague in the Harbin area during the second Manchurian epidemic. In nine cases (eight adults and one child 2 years old) no pneumonic lesions of any sort were seen, but congestion and edema of the lungs, slight reaction in the fauces, larynx, and trachea, and in some cases cervical buboes were observed. They thought that in this group *P. pestis* entered through the respiratory tract, without suggesting the exact site of invasion, and gave rise to plague septicemia without true pneumonia. To make allowance for this possibility, they suggested the term "pulmonary plague," but this directs attention to the lungs rather than the upper part of the respiratory tract. In three of nine autopsies in the Los Angeles epidemic, pulmonary consolidation was not seen but there were hemorrhagic edematous lesions in the tonsils, epiglottis, and vocal cords.

One further distinction must be made: It is conceivable that this form of plague could also arise from flea bites on the neck.

An explanation of this unusual form and the better known pneumonic form began with the

work of Wells and Wells (62) and many others on aerial infection with droplets and droplet nuclei. The wide differences in behavior between large and small droplets is obviously significant in these forms of plague. The term "droplet infection" is rarely, if ever, mentioned, even in the current plague literature. The classical experiments of Jorge in 1905 and Strong and Teague (58), exposing petri dishes at varying distances from a coughing pneumonic plague patient, measured only coarser particles. On first thought, it might seem that such particles would enter the respiratory tract and be deposited at resistant sites as in other bacterial pneumonias. But the upper part of the respiratory tract is furnished with no known defense against *P. pestis*. The smaller the particles the more likely they are to be inhaled and lodge in the highly susceptible lung itself.

Given a heavy concentration of organisms per unit volume of air and prolonged exposure in this pestilential air, inhalations of particles of different sizes begin the invasion through the deepest parts of the respiratory tract, the bronchioles, and pulmonary alveoli. Perhaps only the larger particles can lodge in the upper respiratory tract and give rise to tonsillar or to septicemic plague.

So far as the influence of climate is concerned, the California outbreaks cast doubt on the idea that pneumonic plague occurs only in cold weather when a low water deficit of the atmosphere presumably enables the sputum droplets to float in the air for a considerable time. The disease has been observed in countries with warm or even hot climates where these conditions do not exist. The circumstances favoring aerial infection are immediate proximity to the infector, overcrowding, and diverse social factors. Customs as, for example, in Los Angeles where a prolonged burial ceremony was held with many people attending, promoted exchange of the bacilli.

Until quite recently the diagnosis of pneumonic plague was virtually a death sentence; the mortality rate was nearly 100%. Until 1946, only nine recoveries were reported (60). Even in the past, however, isolation of the patients and segregation and careful observation of their contacts shortened the outbreaks. Prophylactic chemotherapy of contacts with sulfonamides in Madagascar and in other areas with tetracyclines and prompt treatment of the patients with streptomycin or tetracyclines arrested the spread of a pneumonic outbreak in progress for 3 weeks (17, 64).

Experimental Plague Induced through Respiratory Tract

Ever since the pathobacteriological diagnosis of human primary pneumonic plague in December 1896 by Childe (6), numerous attempts have been made to reproduce this form of plague in experimental animals and success has varied. The first experiments were on primates, but it seems better to begin with those on laboratory rodents.

Guinea Pigs

Intranasal and intratracheal infection. In 1899 at the Institute Pasteur, Batzaroff (3), on the advice of Roux, introduced a cotton swab charged with virulent plague bacilli into the nostrils of guinea pigs and reported that this produced a bronchopneumonia which, according to Nattan-Larrier and Richard (39), resembled human primary pneumonic plague. This mode proved not always so successful in the hands of other experimenters. Kolle (25) modified it by instilling the bacilli into the buccal cavity, the nose, or the conjunctival sac. Only a few guinea pigs got pneumonia; the others died from cervical bubonic plague and septicemia. Either the direct intratracheal inoculation of a suspension of 50,000,000 *P. pestis* (2) or intranasal instillation of a suspension of virulent bacilli into the nostril of guinea pigs anesthetized with barbiturates (35) has produced a model of pneumonic plague. From 200 to 700 *P. pestis* are flushed into the deeper respiratory passages by this latter simple technique. This experimental lung infection, with its broncholymphatic, pneumonic, and terminal septicemic phases, resembles the human type histologically. Intranasal infection is considered adequate for testing immunization and treatment (13, 37). Some guinea pigs infected by these methods have transmitted the infection to normal cagemates. This is not usual, however, even if the animals are fed wet carrots and cabbage, as stipulated by Batzaroff.

Bacterial clouds. Under the influence of studies on pulmonary tuberculosis, Martini (31, 32) and subsequently Strong et al. (59) exposed guinea pigs to sprays of suspended plague bacilli in specially constructed glass cages. Over 25% of the animals so exposed had lesions of pneumonia at autopsy; the remaining 75% had cervical

adenitis, tracheobronchitis, and rapidly fatal septicemia. The only explanation that could be offered at that time was that superficial inspiration and the position of the larynx of the guinea pig prevented the organisms from penetrating into the bronchioles and air sacs. Once the influence of aerosol particle size on respiratory infections was known, Druett and his associates (10) conducted careful studies and explained the two forms of plague originating in the respiratory tract of the guinea pig. They develop according to the size of the infected particles presented to the host.

Particles smaller than 1 μ initiate bronchopneumonia, which leads to septicemia and death. Large particles, 10 to 12 μ in diameter, apparently deposited in the region of the head, penetrate the local epithelium and, through the afferent lymphatics, lead to septicemia much sooner than when organisms are deposited on the bronchial or alveolar wall. Septicemia arising from the primary focus in the cervical lymph nodes caused infection of the lung but no pneumonia.

The respiratory tract of the guinea pig (a nose breather) prevents particles larger than 4 μ from reaching the lungs, and in neither form of the disease is the cellular picture that of primary pneumonic plague in man. In this respect the pneumonic lesions produced by this method differ from those after intranasal or intratracheal inoculation.

The British workers also made cross-infection studies. Twelve animals exposed to 7 LD_{50} of single organisms were housed with 12 controls. Cross infection occurred in about 18% of the control animals. Although erratic, the infections were more likely when the initial disease was primary pneumonia. Interestingly, the cross-infected animals suffered from the form characteristic of that following exposure to large particle clouds, i.e., septicemia but no pneumonia. Since this form is only occasionally contagious, it cannot establish epizootics.

Several Russian workers, Korobkova (27), Y. N. Rall, and V. P. Smirov (*cf.* Pollitzer (46)), also tried but failed to produce primary pneumonic plague in guinea pigs as well as in sisels and marmots by inhalation. Their contact infections were probably attributable to the same type of infection as that observed by British workers. The particle size used in these experiments is not given in the papers.

The guinea pig is unsuitable for experimental epidemiological study of primary pneumonic plague, but since the respiratory infection is usually much more severe than the cutaneous or subcutaneous infection, it is most useful in the evaluation of vaccines and the study of immunity.

Mice

Experimental respiratory infection in the laboratory mouse has been achieved either by exposure to aerosols (19, 31, 51–53) or by intranasal instillation. Animals so infected have been sacrificed at intervals and the development of the pneumonia has been followed in serial microscopic sections.

Inhalation of infected particles produced lesions quite similar to those of human primary pneumonic plague. The process began in the alveolar septa with intense cellular infiltration, followed by massive hemorrhage and edema, bacterial multiplication in the blood vessels, and progressive extension into adjacent lobules of lung tissue.

After instillation, the early lesions were in the bronchi, and peribronchial masses of bacteria appeared before cellular infiltration in the alveoli. As the inflammatory reaction progressed, the pneumonic lesions at the time of death resembled those of human primary pneumonic plague.

In the study of immunity and chemotherapy, the mouse is adequate, but few attempts to accomplish cross infection have succeeded. Occasionally a contact mouse, exposed to a group of six infected by inhalation, would die of septicemia with a few isolated pneumonic infarcts.

In the early inhalation experiments by Martini (31) 19 mice were exposed to infective spray but only 4 that died had lesions of pneumonia. The spray, generated with a Paroleine sprayer (Burroughs Wellcome), consisted of particles of various sizes. Meyer and Larson (38), in numerous experiments using a Wellstype atomizer, exposed large series of mice in closed chambers for 3 min. to clouds containing 800,000 to 1,600,000 plague bacilli per liter. These particles were also of different sizes. The infections resulting from these exposures were variable. Some 65% of the mice died with lesions of lobular lobar pneumonia, 17% with cervical buboes and pneumonic infarcts, 14% with cervical buboes, and 4% with septicemia and no visible buboes. In other tests, over 60% died with lesions of septicemia and cervical buboes, only 20% with

primary pneumonia. Probably the particle sizes differed in the different experiments. Applying the observations of Druett and associates (10), the large particles impinged on the mucosa of the nose and upper respiratory tract and gained entrance into the lymph channels leading to buboes in the cervical lymph nodes, or they invaded the blood stream without primary localization in lymph tissue.

Mice with this form of plague are poor infectors. They too are nose breathers. Their respiratory tract successfully prevents large particles exhaled by the animal with pneumonia from reaching the lung of the contact, so a continuous infection chain cannot be maintained. The mouse is not satisfactory for study of pneumonic plague.

Other Rodents

Plague has been produced in small and large marmots (*Arctomys bobac, Spermophilus citellus*) by exposing them in a special wooden box for 5 to 10 min to a spray of *P. pestis* in saline (22, 65, 66). The mortality rate was 100%, but only 5 of 13 tarabagans and sisels suffered from primary pneumonic plague, the remainder from primary septicemia. This resembles the type of disease observed in Manchuria in 1920-1921, with involvement of the tracheobronchial lymph nodes and few lesions in the lung parenchyma. When the marmots were kept in a central compartment and were surrounded with cages holding contacts, those with pneumonia did transmit the septicemic type. The incubation time was short. Exposure to the marmot infected by inhalation was continuous since the wire netting holding the infector and the contacts allowed aerial transmission. Nevertheless, transmission was quite variable. In one experiment three of ten; in another, after exposure for 4 to 6 days, seven of nine contacts contracted plague; the report does not state whether all the contacts contracted primary pneumonic or bubonic septicemic plague. The latter had not been recognized in 1916 when the experiments were made. According to recent reports by Russian workers (Rall, 1958; Smirnov, 1956; *cf.* Pollitizer (46)) primary pneumonic plague did not develop in marmots or sisels kept in close contact with ainmals suffering from secondary plague pneumonia.

Experiments with rabbits have given contradictory results. Batzaroff (3) and Calmette and Salimbeni (5) had successful results with nasal swabbing. Tsurumi (61) claimed success with various methods. At the Hooper Foundation, pneumonic lesions have been produced by inoculating directly into the lung with a syringe, a procedure originally used by Shibayama on guinea pigs. Significant is the statement by Martini (31) that not pneumonic plague but septicemia results when rabbits receive *P. pestis* by inhalation.

Monkeys

Shortly after Childe's work, studies to reproduce pneumonic plague in experimental animals were undertaken, and it is probably not coincidence that primates were first chosen. Three separate instances of spontaneous plague outbreaks in monkeys had just then been observed in India by Clemow in 1900 (7). Commenting on the propagation of plague in Hurdwar, Hankin (18) described his observations on a primate that died shortly after it had been brought to his laboratory. Nasal secretions contained innumerable bacilli that looked like *P. pestis*, and they produced plague in rats. Subsequently the organs of the primate yielded the plague bacillus, but unfortunately an autopsy report is not available. After having seen the intolerable conditions under which monkeys were living during the epidemic, Hankin suspected that inhalation of dust infected with *P. pestis* caused the fatal infection in the monkey.

Experiments with monkeys of different genera and species have been undertaken since, and the individual susceptibility to parenteral infection has varied.

Intratracheal instillation. The Austrian Plague Commission reported that primary pneumonic plague developed in a monkey which took a deep breath after a suspension of *P. pestis* had been introduced into its mouth (1). Wyssokowitz and Zabolotny (69), Polverini (47), and Zabolotny (70) easily produced the pneumonic form by introducing suspensions of plague bacilli with the aid of a catheter into the trachea of anesthetized primates.

To evaluate antimicrobial drugs or vaccines, McCrumb, Larson, and Meyer (33) and Ehrenkrantz and Meyer (11) administered a diluted, agar-grown culture by direct laryngoscopy through a hard rubber catheter into monkeys under barbiturate anesthesia. Pneumonic infec-

tion was established with as few as 120 to 270 organisms in *Macacus rhesus* and *Cynomolgus philippinensis*. Individual variations were overcome when 1,000,000 to 100,000,000 were instilled. The monkeys were asymptomatic for 1 to 3 days. Fever was the first indication of active infection. Roentgen evidence of pneumonitis appeared soon after the onset of fever, and the pneumonitis progressed. The infection terminated fatally on the fifth to eighth day, usually accompanied by septicemia.

This method was chosen because it made certain that the organisms reached the lung; it required no complicated equipment and had been used by the most qualified experimental pathologist, L. Schmidt, in his extensive studies on the chemotherapy of experimental tuberculosis. It was not chosen to study the details of the development of pneumonic plague nor to illuminate the mode of introduction. It has obvious shortcomings. Anesthesia may disturb pulmonary function, adding a provocative factor. Intubation may localize the lesions or create a portal of entry. The inoculum in a fluid medium might for some time supply the organisms with a pabulum unapproachable by the defense mechanism of the host.

Nevertheless, the gross and microscopic lesions produced by the intratracheal and inhalation procedures are quite similar except that by tracheal instillation the organisms are introduced into the bronchi and terminal bronchioles rather than the air sacs. Thus the genesis of the lesions is the same as that in guinea pigs infected by intratracheal instillation.

Inhalation. In an effort to learn the pathogenesis of pneumonic and primary septicemic plague, Strong and Teague (58) put 55 Philippine monkeys in closed glass cages. They sprayed virulent plague bacilli suspended in saline for 2 to 3 min into the air the monkeys breathed. All the animals so exposed contracted plague. Unfortunately details are not given, but the statement that "the primary point of infection may be not only the lungs but also the mucous membranes of the mouth and throat" suggests the possibility of the nonpneumonic form. Under the influence of Koulecha's view (29) that pneumonic plague is primarily septicemia and that the lungs become secondarily involved by way of the blood, they searched for a portal of entry in the upper respiratory tract. They found changes in the tonsils only if the bacilli had been placed on the posterior part of the throat with a glass rod. But in a final statement they concluded that primary septicemia sometimes takes place and death may occur, although rarely before lesions are visible in the lungs or lymph nodes.

By exposing unanesthetized primates to infectious aerosols with measured doses in a special exposure apparatus, Speck and Wolochow (55) studied experimental pneumonic plague in *Macacus rhesus*. The LD_{50}, determined here for the first time, was about 20,000 inhaled cells. The clinical and laboratory findings were similar to those of others (11, 33).

Incidental to their work with guinea pigs, Druett and his colleagues (10) made some experiments with monkeys, using single-organism clouds and particles 12 μ in diameter. Both produced lobar pneumonia although the disease took longer to develop after exposure to large particles.

Contact cross infection. At the International Plague Conference in Mukden and in his book, Zabolotny (71) noted as early as 1897 that monkeys given Haffkine or other vaccines contracted lung infection when left in the same cage with infected animals. One monkey treated prophylactically with antiplague serum also became infected when similarly exposed.

Girard and Robic (15) and Robic (48, 49) succeeded in a single experiment in bringing about cross infection among a group of nine "maki," Madagascar lemurs (*Propithecus* species). Three lemurs were inoculated by the conjunctival or nasal route with two drops of a broth culture of virulent *P. pestis*. On the third day thereafter, two of the animals died, and on the fifth day the third died, all with lesions of typical pneumonic plague. Of the six lemurs in close contact in the box with the infected, five died within 4 or 5 days, invariably within 24 to 48 hr after showing signs of illness. None coughed but three of the five had mucopurulent nasal discharge teeming with plague bacilli that proved highly virulent for guinea pigs. Dissection of the neck disclosed enlarged hemorrhagic cervical and submaxillary lymph nodes and septicemia. The sixth lemur escaped the cross infection; although it was held in the contaminated cage, it did not acquire plague. Robic considered the infection in the contacts to be a form of "peste pulmonaire" serially transmitted by the infective mucous

discharge. The upper respiratory tract or the eyes may have served as the portal of entry. This experiment is also valuable in that it shows the high susceptibility of lemurs to plague.

At the Hooper Foundation (38) healthy primates were exposed to cagemates with frank clinical pneumonia produced by the same method as that used by McCrumb and co-workers (33). In several experiments monkeys with fever and definite roentgenographic evidence of pneumonia were placed in a cage divided in two compartments which made bodily contact impossible but allowed free passage of the exhaled air. The exposure time ranged from less than 1 day to the time the infected primate survived after the healthy one was introduced.

In 36 exposure experiments, 23 of the contacts died of plague, on the average 7 to 7½ days after the beginning of exposure. Nineteen contracted cervical bubonic septicemia and only three developed pneumonic plague. Monkeys exhaling plague bacilli were selected for the experiments. Blood agar plates were held 4 to 5 in. from the monkey's mouth for 30 to 60 sec. In every instance in which the plates contained over 20 organisms, the contact monkey contracted plague. The contagiousness was roughly correlated with the number of bacilli exhaled and total exposure time. Most of the infectors were severely ill and most of them coughed, but the extent of these indications was not measured because it was impossible to observe the animals all the time.

Autopsies were exceptionally thorough. In the absence of pulmonary consolidation, the entire upper respiratory tract was dissected. Two forms of plague originating in the respiratory tract were seen. Nineteen contracted cervical, bubonic, septicemic plague, and three developed pneumonic plague with no lesions in the upper respiratory tract. Serial sections indicated that the plague bacilli had entered the upper respiratory tract through the lymphatic tissues of Waldeyer's ring in the oropharynx. The afferent lymphatics carried them to the regional lymph nodes and induced primary buboes, and septicemia followed rapidly. This form was not contagious. Pneumonic plague developed after inhalations of exhaled particles into the tissues of the terminal bronchioles and alveoli. Only one contact with primary pneumonia passed on the infection to another contact, but here again it took the cervical, bubonic, septicemic form and the chain was broken.

The influence of particle or droplet size and whether the nose-breathing primates received the infective dose through the nasal passage remain to be learned. The normal clearing mechanism would bring the organisms into the oropharynx and in contact with lymphatic tissues well suited for multiplication of plague bacilli.

These observations are disappointing because they indicate that an epizootic, pneumonic form of plague can probably not be reproduced in primates. On the other hand they explain some observations in the Los Angeles epidemic and the so-called nonpneumonic or pulmonary type of lung pest recorded by Wu Lien-Teh et al. (67) and by Pollitzer and Li (45) in 1920-1921 in Harbin.

Probably the entire respiratory tract of man can serve as a portal of entry for plague bacilli in the pestilential air.

Immunization

At the International Plague Conference in Mukden in 1911, Zabolotny reported some experiments on monkeys, summarized in the statement that these animals could be protected against lung infection only by repeated high doses of agar-grown vaccines. In his opinion, it was much more difficult to vaccinate against respiratory than bubonic plague. Strong and Teague (58) later vaccinated large numbers of guinea pigs and monkeys by subcutaneous injection of a living, attenuated plague culture and later exposed them to infection by inhalation; an equal number of unvaccinated and vaccinated animals were exposed at the same time in each series. Of the vaccinated guinea pigs, 75% survived the exposure; many of the unimmunized animals died not of primary pneumonia but of primary bubonic infection of the cervical nodes and of secondary septicemia and sometimes secondary pneumonia. Only very few died of primary pneumonic plague. Nonimmunized monkeys exposed to inhalation almost all died of primary pneumonic infection. Only about 10% of the vaccinated animals survived infection by inhalation. A committee of that Conference summarized its opinions: (i) that the statistical evidence points to the conclusion that some degree of protection is conferred against bubonic plague by the use of vaccines, (ii) but experience during the 1910-1911 epidemic does

not allow any definite conclusion about the value of prophylactic inoculation against plagues pneumonia, and (iii) that inhalation experiment) on animals (guinea pigs, white rats, monkeys should be carried on to find out which vaccine can best be used against pneumonic plague.

These conclusions have been amply confirmed in the course of mass vaccination with living attenuated plague vaccines in Indonesia (41) and Madagascar (16). An appreciable immunity was obtained when vaccination was repeated.

Living or killed organisms or antigen in aqueous suspension customarily administered by the subcutaneous or cutaneous route in two inoculations have rarely protected more than 10% of the animals against inhalation infection. Repeated inoculations of killed organisms or envelope antigen with alum synergist or adjuvants have protected 90 to 100% of guinea pigs and monkeys against inhalation infections. In the course of these experiments, marked lung involvement was regularly found in the absence of spleen and liver lesions when partly immune animals died from the pulmonary challenge after prolonged illnesses.

Pokrovskaia and Kaganova (43) and Pokrovskaia et al. (44) attributed this lack of immunity to "a weakly developed reticulo-endothelium and to little permeability for antibodies of the lung tissues" and attempted to increase the production of clasmatocytes by causing the animals to inhale living vaccine strains (E.V. of Girard and Robic and a Russian strain A.M.P.). These and other Russian workers (26, 28) combined repeated respiratory immunization and a subcutaneous inoculation and reported high resistance to cloud infection with virulent plague bacilli in small series of guinea pigs and monkeys. Three subcutaneous inoculations of the live vaccine protected 85.7%, and four inhalation vaccinations prevented pneumonic plague in 66.7% of the guinea pigs. The doses of organisms used in challenge infections were large: 25,000,000 for the nasal instillation, 250,000,000 for the inhalation.

At the Hooper Foundation such experiments on guinea pigs and mice yielded similar results.

Any systemic immunization with living or killed vaccines that have produced solid immunity against bubonic infection has conferred appreciable resistance against respiratory infection. Formalin-killed virulent plague bacilli coated with alum or incorporated in an adjuvant given intramuscularly in two doses, followed by one booster dose 1 to 3 months later, has stimulated antibodies and complete resistance against an inhalation infection in experiments at the Hooper Foundation and elsewhere (30). Spacing of individual inoculations and the total period of vaccination are at least as important as the total dose of the immunizing agent. Active immunity against pneumonic plague has been attained with live or killed adjuvant vaccine if the basic immunity stimulated by two inoculations 30 days apart is intensified by a subcutaneous booster inoculation in the 19th week, to such an extent that most of the vaccinated primates have survived an intratracheal infection with 40,000 virulent *P. pestis*; the controls succumb within 4 days with clinical lesions of pneumonic plague. One of six vaccinated primates showed shadows in the thoracic roentgenograms and slight elevation of temperature, but it survived.

In experimental studies (i) local immunization of the respiratory tract has not been required to protect guinea pigs and primates against pneumonic plague, (ii) the necessary immunity has been obtained by intramuscular injection of killed or live plague bacilli provided they are coated with alum or incorporated in adjuvants and followed by a booster inoculation, (iii) vaccines in aqueous suspension have produced a low-grade immunity, (iv) active immunity against pneumonic plague has not been induced with two inoculations within 2 weeks, (v) the basic immunity obtained with adjuvant vaccine has had to be reinforced with a booster shortly before exposure, (vi) the immunity has declined after 3 months, and to revive it, systematic reinoculation has been required, and (vii) the state of immunity has been shown in serum antibody levels. Present procedures of immunization of man against plague with aqueous vaccines, according to serum antibody determinations, are essentially ineffective against pneumonic plague.

Experimental pulmonary plague, whether secondary to bubonic plague or primary pneumonic plague, may well serve as a perfect model to elucidate the pathogenesis of this unique airborne infection, particularly if the infection and disease could be consistently reproduced. Various species of primates should be tested to learn which is most suitable for animal-to-animal passage. The biophysical laws governing exhalation of *P. pestis* in sputum droplets and transfer to

new hosts could then be learned. It is reasonable to suspect that the half-life of the plague bacilli in sputum droplets may differ from that in artificial moist particles created by new instruments from suspensions of cultured organisms or tissue slurries. A blood-containing excretion consisting of enormous masses of living and dead bacilli probably contains toxic substances that may influence the deposition in the upper or mucociliary escalator of the lower respiratory tract.

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